action.

There is appended hereto as Appendix A a copy of Claims 33-40 and 42-54 wherein deletions made in response to the action are shown by brackets [ ] and added matter is underlined.

II. The Claims

A. Response to the Claim Objection

Claim 50 has been amended to correct the inadvertent misspelling of the word "containing"

B. Response to the 35 U.S.C. 112 Claim Rejections

Claim 33 has been extensively revised , both for clarification purposes and in an earnest effort to respond to the Examiner's criticisms.

More particularly, since the immunoassay method set forth in Claim 33 and other claims herein is specific to the detection of the presence of the cell wall C-polysaccharide antigen common to all serotypes of *Streptococcus pneumoniae*, and that antigen has been found to be detectable whether present in a liquid sample in a free state or present within bacterium, this claim and the others presented now recite that the method detects "the presence of the cell wall C- polysaccharide antigen of *Streptococcus pneumoniae*". It is to be understood that this presence in mammalian fluids, when coupled with clinical observations of the symptoms and conditions of an infected patient, permits speedy and confident diagnosis of a disease known to be caused by *Streptococcus pneumoniae*, and particularly diseases such as pneumonia, otitis media and meningitis.

Claim 33 now recites steps of providing a sample, a detection reagent and detecting the

presence of a signal indicative of the presence of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*, which in turn is indicative of the presence of *Streptococcus pneumoniae*. The Examiner's gratuitous assertion that "The antibodies purified over the column contained not more than 10% protein and would bind antibodies to *Streptococcus pneumoniae* antigens that are not C-polysaccharide binding antibodies"(Office Action, p.3) is contrary to Applicant's experience and appears to be an unsupported opinion of the Examiner. Applicants have not tested their antibodies for protein content nor urged that this parameter of antibodies has any relevance to this invention. What they do urge, have demonstrated in their application and rely upon here is that the antigen separated from a culture of whole *Streptococcus pneumoniae* bacteria coupled to a column through a spacer molecule, which column is used to affinity purify polyvalent antibodies to *Streptococcus pneumoniae*, contains not more than 10 % protein.

It is noted that Applicants' preferred immunochromagraphic (ICT) assay for the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* received FDA approval in the year 2000 and is the first and only such test for *Streptococcus pneumoniae* in humans to have been so approved to date. Moreover, Applicants' assignee has by now sold hundred of thousands, at least approaching and perhaps exceeding a million of these preferred ICT tests which have been successfully used in rapid patient diagnosis by or at the behest of countless health professionals around the world. Section (e) of claim 33 no longer contains the terms criticized in the Office Action, including "essentially comprises"., and "may in part be conjugated to a tag". This part of the claim now recites an immunoassay process for detecting the target antigen which is the C-polysaccharide cell wall antigen of *Streptococcus*

pneumoniae .

Claim 34 has been amended to recite that it depends from Claim 33.

Claims 35, 37 and 38 now correspond to Claim 33 in reciting that the sample is "liquid".

Claims 40 and 49 have been amended to delete the word "developed" before meningitis. It is noted , however, that the term "developed" was intended to refer to the known fact that human patients who initially are found to have pneumonia or another *Streptococcus pneumoniae* - caused disease do from time to time develop meningitis. There was no intention to create any new category of meningitis.

Claim 41 has been canceled inasmuch as part (e) of Claim 33 has been amended to recite an immunoassay process.

Claim 42 is believed proper as now presented. Immunochromatographic methods are well-known in the art. The essence of Applicants' invention is providing antibodies that reliably detect the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* and Applicants are entitled to cover using those antibodies in any immunochromatographic process falling within the scope of step(e) of Claim 33.

Claim 43 has been extensively revised and is believed to clearly recite details of a preferred ICT process.

Claim 45 has been amended to depend upon Claim 44, and as such, to constitute a specific embodiment of Claim 43 wherein the sample is human urine. This amendment is believed to obviate the Office Action's criticism of Claims 46,48 and 49.

Claim 50 has been amended to explain that when a line of color appears along the immovably bound stripe of antibodies that have been purified as disclosed and claimed, it

denotes the presence in the sample of the target C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*.

Claim 51 has been clarified by the amendments to Claim 50. The apparent "color change" previously recited involved the change along the stripe of immovable antibodies from a more or less white color to the characteristic color produced by finely divided gold as a result of the massing of the gold along that line. However, Claim 50 now explicitly recites what takes place along the line and the expression "color change" no longer appears in that claim. Claim 52 now depends from Claim 50 and is believed to be clear.

Claim 53 depends on Claim 52 and Claim 53 depends on Claim 54, but since the dependency of Claim 52 has been corrected, the criticisms of these claims (Office Action p.7, second paragraph) no longer apply.

Insofar as Claim 52 is concerned, its dependency on Claim 50 imparts the antecedents the Examiner alleged to be missing. Moreover, the term "entrained conjugate" has been replaced by language that makes clear that liquid sample and conjugates of label and antibodies are allowed to flow together along the test strip.

III. Response to the 35 U.S.C. 102 Claim Rejections

These rejections, which overall are directed only to one or more of Claims 50 to Claims 54 are believed to be obviated by the claim amendments made herein. In this regard, the inadvertent clerical errors which resulted in incorrect claim dependencies which prompted some of these rejections are regretted.

Notwithstanding the amendments herein, which clearly obviate most, if not all, of these

rejections, it is believed that further discussion is in order here.

As noted in M.P.E.P. 2131, "TO ANTICIPATE A CLAIM, THE REFERENCE MUST TEACH EVERY ELEMENT OF THE CLAIM"

None of Claims 50-54 are anticipated by Imrich or May et al, either literally or inherently because each of claims 50-54 requires that the antibodies in both zones of the ICT device be antibodies which have been affinity purified with the antigen containing not more that 10% protein obtained as set forth in method steps (i) - (viii) of Claim 50, which antigen is then coupled to an affinity column through a spacer molecule and over which polyvalent antibodies to *Streptococcus pneumoniae* are then passed.

No cited reference describes or discusses these specific antibodies, which are the essence of Applicants' invention.

Moreover, neither Imrich nor May et al recognize that when utilizing Applicants' unique antibodies, it is advantageous to employ them in both the first and second zones of the ICT strip in order to capture the cell wall-C-polysaccharide antigen of *Streptococcus pneumoniae* which has multiple determinants reactive with these antigens.

Applicants, moreover, do not claim to have invented the ICT device, the ICT strip, or any specific label, including finely divided gold. However, Applicants, having invented particularly sensitive antibodies for the detection of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*, are entitled to claim its use in the preferred ICT assay form in which their assignee sells it in commerce and have chosen to do so.

IV. Response to 35 U.S.C. 103 Rejections

The 35 U.S.C. 103 rejections overlook that the statute requires that the references supporting such a rejection be of a caliber such that "the invention as a whole" would be obvious to a person of ordinary skill in the apposite art–in this instance, immunology.

The rejection based upon Imrich in light of Gribnau in view of Krook is not well taken. The primary reference, Imrich teaches nothing about any specific antibody for an antigen of *Streptococcus pneumoniae* and it is not at all clear from the Imrich et al disclosure and claims that Imrich was in possession of an immunoassay that was effective in detecting *Streptococcus pneumoniae* despite the references to it in Claim 15 and in line 2 of column 7. Group A streptococcus, the subject of both Imrich examples and also mentioned in Claims 5 and 19 is different from *Streptococcus pneumoniae*, and so is Group B *streptococcus* likewise referred to in Claim 5 and apparently intended to be referred to in Claim 19.

The mere fact that the Imrich claims refer, very vaguely to "immunoglobulin that specifically binds to the analyte" in each of cl aims 2-4, and 11 and to "immunoglobulin... specific for the microbial antigen selected from various bacteria, including *Streptococcus pneumoniae* antigen" in Claim 13 does not lead one to an operable, useful test of high sensitivity and specificity for detecting an antigen characteristic of *Streptococcus pneumoniae*. After all, "*Streptococcus pneumoniae* " is known to contain several antigens and it is important to know which one is being tested for as well as what antibodies are being used to detect it, neither of which Imrich provides any information about.

Combining Imrich with Gribnau, incorporated by reference in Imrich, gives no information except to suggest that the antibodies for an antigen of any of the bacteria that are discussed by Imrich could be gold-labelled if a workable assay were arrived at. Gribnau,

however, gives no reason to select gold as a labelling agent, suggesting instead that "Sols of dispersed dyes have advantages over metal sols (e.g. gold) in colorimetric assays" (col. 2) lines 45-46. Krook et al discusses an ELISA test, but not a test performed immunochromatographically. It involves the use of two different antibodies, a monoclonal antibody to phosphorylcholine said to be a component of the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae,* and an "affinity-purified polyclonal antibody" which is described in greater detail in the Sjogren and Holme article cited by the Examiner. The antigen used for affinity purification was obtained from *Streptococcus pneumoniae* bacteria by the phenol-water extraction method and contained 17% protein, whereas Applicants' antigen is obtained from a bacterial culture by a wholly different method recited, inter alia, in Claim 50 and contains a maximum of 10% protein.

Applicants' polyclonal antibodies, raised against whole bacteria, are affinity-purified over a column to which Applicants' purified antigen is coupled through a spacer molecule, whereas the antigen used for affinity purification by Krook was mixed with pieces of DEAE Sepharose and incubated, with further mixing, for 48 hours. There are many reasons to believe that antibodies passed over such differently prepared columns would be quite different, since different determinants are likely to be stripped from them in different proportions.

Certainly there is no reason to suspect that Applicants' ICT immunoassay, which is highly sensitive and specific, and prima facie different from Krook's ELISA assay, could, much less would, be arrived at by combining the Imrich, Gribnau, Krook and Sjogren references

It is noted that Applicants' assay as described and claimed does not require diluting a

sample before assay, filtration of the sample before analysis or extraction of the sample prior to analysis.

The reference combination suggested by the Examiner simply does not approximate, let alone meet, the requirement to show obviousness of Applicant's invention as a whole and should hence be withdrawn.

The rejection of Claims 50 and 51, as now amended, over May et al in view of Krook et al is equally inadequate under U.S.C. 103 to demonstrate obviousness to those of ordinary skill in immunology of Applicant's invention as a whole.

May et al discloses a type of ICT device that is often referred to as a "complete immersion" device, which was originated for the purpose of home pregnancy tests, where the device was to be held in a flowing stream of urine in order to apply the test sample to the device. Such devices, in use, have many limitations and drawbacks in comparison to Applicant's preferred device utilized in its commercial embodiment and covered in Claim 50. It is again noted in this regard that Applicants do not contend they invented this device per se. but only point out that they have chosen it for their commercial, FDA-approved, embodiment. May et al suggest no assay for any antigen of *Streptococcus pneumoniae*. Their vague reference to a "multiple analyte test for the detection of the presence of different serotypes of *Streptococcus*" which "can be prepared for groups A, B, C and D"(p.19) has nothing to do with the present invention and certainly does not render obvious to a person of ordinary skill "the invention as a whole" covered by any of the present claims.

Krook has been discussed above and its use of the monoclonal and polyclonal antibodies described in Sjogren, does not lead to Applicants' purified antibodies which

Applicants employ both in the first and second zones of its assay. It is noted that this antibody combination is also described earlier in Holmberg et al, of which Krook and Sjogren are both co-authors.

ADDITIONAL COMMENTS

The additional voluminous references cited by the Examiner have been considered. They neither anticipate nor render obvious Applicants' purified antibodies obtained by Applicants' methodology nor Applicants' assay in which they are employed.

CONCLUSION

It is believed that this application is now in condition for allowance and early action to that effect is accordingly requested.

Respectfully submitted,

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APPENDIX A

33 A method of detecting the presence of the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, in a liquid sample, which method comprises the following steps:

a) culturing *Streptococcus pneumoniae* bacteria, to obtain a desired size of culture and harvesting therefrom cells thereof as a wet cell pellet;

b) separating from the wet cell pellet the cell wall C-polysaccharide antigen containing not more than 10% protein by performing a series of steps which comprises;

(i) suspending the wet cell pellet in an alkaline solution and mixing;

(ii) adjusting the pH to an acid pH with a strong acid;

(iii) separating the mixture from step (ii) into two layers;

(iv) removing the upper layer and adjusting its pH to approximate neutrality;

(v) adding to the product from step (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi) adjusting the pH of the product from step (v) to an alkaline pH with a weakly alkaline aqueous solution; and (vii) separating out the cell wall C-polysaccharide antigen containing not more than 10% protein;

c) coupling to a chromatographic column through a spacer molecule the cell wall C-polysaccharide antigen containing not more than 10% protein obtained in step (b);

d) passing polyvalent antibodies to *Streptococcus pneumoniae* over the

chromatographic affinity column of step (c) to produce purified antigen-specific antibodies; and

e) conducting an immuno-assay upon a liquid sample suspected of containing *Streptococcus pneumoniae* and/or its C-polysaccharide cell wall antigen which immuno-assay comprises the [step] steps of [detecting the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*, if present, by contact of the liquid sample with a detection agent which essentially comprises purified antigen-specific antibodies from step (d) hereof which may in part be conjugated to a tag,]

(i) contacting the liquid sample with conjugates of purified antigen specific antibodies from step(d) hereof and a labelling agent capable of manifesting a color or a detectable signal upon completion of the immunoassay, whereupon C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in the sample, whether or not in free form, will react with said conjugates to form labelled antibody-antigen conjugates,

(ii) further contacting the liquid and all of the conjugates it contains with a solid surface upon which a mass of unlabelled antigen-specific antibodies from step (d) hereof have been immobilized, whereupon any labelled antibody-antigen conjugates present will react with the immobilized antibodies on the surface to form labelled antibody-antigen-immobilized

antibody sandwiches, and [whereby that a detectable physical or chemical change is effected as a result of contact of the detection agent with the sample.]

(iii) detecting any label thereby accumulated on the solid surface by a detection means appropriate to the nature of the label so as to confirm the presence of the *Streptococcus pneumoniae* C-polysaccharide cell wall antigen in the sample.

34 The method of claim [32] 33 in which the spacer molecule of step (c) is a protein molecule.

35 The method of claim 33 wherein the sample of step (e) is a natural [fluid] liquid of mammalian origin.

36 The method of claim 35 wherein the liquid sample of step (e) is human urine.

37 The method of claim 36 in which the [fluid] liquid sample is taken from a patient exhibiting clinical signs of pneumonia.

38 The method of claim [37] 36 in which the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

39 The method of claim 35 wherein the liquid sample of step (e) is human spinal fluid.

40 The method of claim 39 wherein the sample is obtained from a patient suspected of having [developed] meningitis.

(41-Cancelled)

42 The method of claim [40]33 in which step (e) is an immunochromatographic

("ICT") process.

43 The method of claim [33] 42 in which step (e) is conducted by

a) contacting a liquid sample suspected of containing *Streptococcus pneumoniae* and/or its free cell wall C-polysaccharide antigen, with [an ICT device comprising a housing containing a strip of bibulous material, which strip has] the sample-receiving end of a strip of bibulous material, which strip is contained within an ICT device comprising a housing and itself comprises

(i) a first zone in which has been movably embedded a conjugate of a labelling agent with purified antigen-specific antibodies obtained in step (d) of claim 33, said labelling agent being selected from among those known to [display] manifest a visible color change upon the formation of a labelled antibody [:] - antigen [:]- fixed antibody reaction product and

(ii) a second zone having fixedly bound thereto a stripe of unconjugated purified antigen-specific antibodies from step (d) of claim 33, which zone is equipped with a window in the housing for viewing [color changes] the appearance of a color characteristic of the massing of the labelling agent upon the formation of the labelled antibody - antigen - fixed antibody reaction product;

b) allowing said liquid sample to flow laterally along said test strip to said first zone where it picks up the movably embedded conjugate of labelling agent and antigen-specific antibodies obtained in step(d) of Claim 33;

c) allowing said liquid sample and said conjugate of antigen-specific antibodies to flow laterally together along said test strip to said second zone while concomitantly reacting to form labelled antibody-antigen conjugates with C-polysaccharide cell wall antigen of *Streptococcus*

pneumoniae, free or combined, present in the sample and

d) within not more than 20 minutes after first contacting the liquid sample with the test strip, observing, through said window in the housing whether a line of color has formed, [indicating the presence in the sample of *streptococcus pneumoniae* or its cell wall C-polysaccharide antigen,] indicative of the massing of said label along the stripe of unconjugated purified antibodies, as labelled antibody-antigen-fixed antibody reaction products are formed.

44 The method of claim 43 wherein the sample is a natural [fluid] liquid of mammalian origin.

45 The method of claim 44 wherein the sample is human urine.

46 The method of claim 45 wherein the sample is taken from a patient exhibiting overt clinical signs of pneumonia or another respiratory tract illness known to be often caused by *Streptococcus pneumoniae*.

47 The method of claim 44 wherein the liquid sample is human spinal fluid.

48 The method of claim 45 wherein the liquid sample is taken from a patient exhibiting clinical signs of otitis media.

49 The method of claim 45 wherein the liquid sample is taken from a patient suspected of having [developed] meningitis.

50 An ICT device for the detection of [*Streptococcus pneumoniae* bacteria] the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae* in a liquid sample, which device comprises a housing [containg] containing a strip of

bibulous material having

a) a first zone in which has been movably embedded a conjugate of a labeling agent and purified antibodies specific to the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, and

b) a second zone downstream of said first zone having immovably bound thereto a [portion] stripe of purified antibodies specific to the same cell wall C-polysaccharide antigen of *Streptococcus pneumoniae*, which zone is equipped with a window in the housing for viewing [color changes] the appearance of a line of color along said stripe, which color is indicative of the massing of the labelling agent along the immovably bound stripe as a consequence of the formation of labelled antibody-antigen-immovable antibody sandwiches, whereby the line of color denotes the presence in the liquid sample of the C-polysaccharide cell wall antigen of *Streptococcus pneumoniae*;

all of which antibodies in both zones are further characterized in that their antigen specificity has been attained by passing polyvalent antibodies to *Streptococcus pneumoniae* over a chromatographic affinity column to which is coupled a spacer molecule conjugated to a purified cell wall C-polysaccharide antigen obtained from a culture of *Streptococcus pneumoniae* bacteria according to the following method:

(i) harvesting cells from the said culture in the form of a wet

cell pellet;

(ii) suspending the wet cell pellet in an alkaline solution and mixing;

(iii) adjusting the pH of the resultant mixture to an acid pH with a strong acid;

(iv) separating the acidified product from step (iii) into two layers;

(v) removing the upper layer and adjusting its pH to approximate neutrality;

(vi) adding to the product from step (v) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vii) adjusting the pH of the product from step (vi) to an alkaline pH with a weakly alkaline aqueous solution; and

(viii) separating out the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* having no more than 10% protein.

51 The ICT device of claim 50 wherein the labelling agent is finely divided metallic gold.

52 A method for detecting [*Streptococcus pneumoniae*] the cell wall C-polysaccharide antigen of *Streptococcus pneumoniae* in a liquid sample which comprises

a) contacting said sample with the sample receiving end of the strip of bibulous material [of] contained in the ICT device of claim [49] 50;

b) allowing the liquid sample to flow laterally to the first zone of said test strip where it picks up the movably embedded conjugates of labelling agent and purified antigen -specific antibodies;

c) allowing the liquid sample and the [entrained conjugate] said conjugates to flow laterally together along said test strip to the second zone thereof,

while the conjugates concomitantly react with cell wall C-polysaccharide antigen of *S. pneumoniae* if present in the sample, whether free or bound, to form labelled antibody-antigen conjugates, and

d) within approximately 15 to 20 minutes after the initial contact of the sample with the test strip, observing through the view window whether a line of color has appeared along the stripe of immovably bound antibodies, which line of color indicates massing of label along the stripe due to reaction of the labelled antibody- antigen conjugates formed in step(c) with the immovably bound antibodies to form "sandwiches", thereby indicating the presence in the [test] liquid sample of *[Streptococcus pneumoniae* and/or its] the cell-wall C-polysaccharide antigen of *Streptococcus pneumoniae*.

53 The method of claim 52 wherein the liquid sample is of natural mammalian origin.

54 The method of claim 53 wherein the liquid [same] sample is selected from among human urine, human sputum and human spinal fluid.